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PT 34 AM

## Claims

1. A method for identifying antifungal agents comprising

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i. incubating, with at least one candidate compound, a fungal GTP cyclohydrolase II polypeptide under conditions allowing the binding of the candidate compound to the fungal GTP cyclohydrolase II; and

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ii. selecting, by step ii), at least a candidate compound which binds to the fungal GTP cyclohydrolase II of step i); or

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iii. selecting, by step iii), at least one candidate compound which reduces or blocks the activity of the fungal GTP cyclohydrolase II of step i); or

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iv. selecting, by step iv), at least one candidate compound which inhibits or decreases transcription, translation or expression of the fungal GTP cyclohydrolase II of step i).

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2. A method as claimed in claim 1, wherein the fungal GTP cyclohydrolase II is encoded by a nucleic acid sequence comprising

a) a nucleic acid sequence shown in SEQ ID NO:1; or

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b) a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO: 2 by back translation; or

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c) a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from a functional equivalent of the amino acid sequence shown in SEQ ID NO: 2, which has an identity with SEQ ID NO:2 of at least 49%, by back translation.

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3. A nucleic acid sequence comprising

a) a nucleic acid sequence shown in SEQ ID NO:4; or

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b) a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:5 by back translation; or

- 5 c) a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from a functional equivalent of the amino acid sequence shown in SEQ ID NO:5, which has an identity with SEQ ID NO:5 of at least 66%, by back translation.
- 10 4. A method as claimed in claim 1 or 2 which comprises testing a candidate compound in a fungal GTP cyclohydrolase II inhibition assay.
- 15 5. A method as claimed in claim 4 which comprises
- 15 a) incubating, with a candidate compound, a fungal GTP cyclohydrolase II in a cell free system;
- 15 b) selecting, by step b), a candidate compound which decreases the activity of the fungal GTP cyclohydrolase II.
- 20 6. A method as claimed in claim 5, wherein the enzymatic activity of the fungal GTP cyclohydrolase II is determined in comparison to the activity of a fungal GTP cyclohydrolase II not incubated with the candidate compound.
- 25 7. A method for determination of GTP cyclohydrolase I or II activity comprising the steps of
- 30 a) adding GTP or GTP analog, NAD<sup>+</sup> and formate dehydrogenase to a sample comprising GTP cyclohydrolase II or I; and
- 30 b) determination of the NAPH content.
- 35 8. A method as claimed in claim 5 or 6, wherein the enzymatic activity of GTP cyclohydrolase II is determined according to claim 7.
- 35 9. A method for identification of inhibitors of GTP cyclohydrolase I or II comprising the following steps:
- 40 a) adding GTP or GTP analog, NAD<sup>+</sup> and formate dehydrogenase to a sample comprising GTP cyclohydrolase I or II;
- 40 b) adding formate, NAD<sup>+</sup> and formate dehydrogenase to a second sample comprising GTP cyclohydrolase I or II;
- 45 c) adding to the sample of step a) and step b) a candidate compound;

- d) determining the activity of both samples;
  - e) selecting candidate compounds that show inhibition in the presence of GTP and no inhibition in the presence of formic acid.
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10. A method as claimed in claim 5 or 6, wherein inhibitors of fungal GTP cyclohydrolase II are identified in an inhibition assay according to claim 9.
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11. A method as claimed in any of claims 5, 6, 8 and 10, wherein GTP is used as substrate and the NADPH content is determined by monitoring the increase in the absorption at 340nm.
- 15 12. A method as claimed in claim 1, 2 or 4 comprising the following steps:
- a) the generation of organisms which, following transformation with a nucleic acid sequence encoding GTP cyclohydrolase II are capable of overexpressing polypeptide with GTP cyclohydrolase II activity;
  - b) the application, to the organism of step a) and to an analogous, untransformed organism, of a candidate compound;
  - c) the determination of the growth, the viability or infectivity of the transgenic and the untransformed organism following application of the substance of step b);
  - d) the selection of candidate compounds, which reduces growth, viability or infectivity of the transgenic and the untransformed fungi following application of the substance of step b).
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13. A method as claimed in claim 12, wherein the organism is a fungus.
14. A method as claimed in any of claims 1, 2, 4 to 6, 8 and 10 to 13, wherein the substances are identified in a high-throughput screening.
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15. A method as claimed in any of claims 1, 2, 4 to 6, 8, 10 to 14, wherein the antifungal agent identified via the method is applied to a phytopathogenic fungus in order to verify the fungicidal activity.
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16. An antifungal agent identified via one of the methods as claimed in any of claims 1, 2, 4 to 6, 8, and 10 to 15.
17. A process for the preparation of a fungicidal composition,  
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- a) identifying a antifungal agent via one of the methods as claimed in any of claims 1, 2, 4 to 6, 8 and 10 to 15,  
and  
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- b) formulating the antifungal agent identified via (a), or an agriculturally useful salt of the active ingredient identified via (a), with suitable adjuvants.
- 15 18. The use of compounds as claimed in claim 16 for producing drugs.
19. A process for the preparation of a pharmaceutical fungicidal composition, which comprises  
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- a) identifying an antifungal agent via one of the methods as claimed in any of claims 1, 2, 4 to 6, 8 and 10 to 15,  
and
- 25 b) formulating the antifungal agent identified via (a), or a pharmaceutically useful salt of the active ingredient identified via (a), with suitable excipients.
20. A method for controlling harmful fungi, which comprises treating the fungi, or the materials, plants, soils or seeds to  
30 be protected from fungal infection, with an effective amount of a fungicidal compound as claimed in claim 16 or a fungicidal composition prepared according to claim 19.
- 35 21. The use of a fungal GTP cyclohydrolase as target for the identification of antifungal agents.

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